STOMATA SIZE AND DISTRIBUTION IN DIPLOID AND POLYPLOID PLANTS

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With plate 205

IN BOTH PLANTS and animals the doubling of the chromosome number in a cell usually results in a corresponding increase in the size of the nucleus and cytoplasmic volume (Wilson 1925). The tetraploid races of *Solanum* examined by Winkler not only had larger stomata and pollen grains than those of the diploid, but, in addition, the chloroplasts were correspondingly larger. More recently Karpechenko (1928) found an increase in stomata size as the chromosome number increased in a series of *Raphanus* × *Brassica* hybrids, although the relationship was not linear. Navashin (1931) also found a rather close correlation between chromatin mass and cell volume in related species and polyploid races of *Crepis*. The relationship between polyploidy and cell size has provided a method for detecting induced tetraploids in *Zea* simply by examining the stomata (Randolph 1932).

In a recent review of autopolyploidy Müntzing (1936) lists fifty-eight cases of polyploidy within species or closely related species. The intraspecific chromosome races are usually differentiated both in morphological and ecological characters. The polyploid forms are generally somewhat larger, more vigorous, tend to be longer-lived, and usually have a different geographical distribution when compared with their diploid ancestors. Although autopolyploidy does not give rise to new species directly, it may be an important factor in the evolution of plant species.

The frequent occurrence of species with diploid and polyploid races, suggests that polyploids may be found in many other supposedly diploid species, as larger numbers of individuals are examined. If the relationship between cell size and chromosome number would permit the use of herbarium material in detecting polyploids, much of the survey work would be simplified. A comparison of diploid and polyploid races is also of considerable interest because the rate of development is decreased in the tetraploid, accompanied by a number of physiological changes.

We have compared the diploid and tetraploid races of *Tradescantia canaliculata* Rafinesque in some detail, and have compared the stomata distribution in diploid and polyploid races or species in both living and herbarium material in other genera. The chromosome races of *T. canali-*

culata were collected by Dr. Edgar Anderson and were grown in adjacent plots in the Arnold Arboretum. The two races are similar in size and are not easily differentiated except by cytological examination. The tetraploid plant of *Secale cereale* L. used in this work was produced from a diploid by subjecting the pre-embryonic cells to extreme temperatures (cf. Dorsey 1936). Stomata counts from living plants in the Arnold Arboretum were from individuals which had been examined cytologically to determine their chromosome numbers.

The volumes of corresponding cells in tetraploid and diploid plants show a high correlation between cell size and chromosome number in *Tradescantia* (Table I). The pollen mother cells and microspores of the tetraploid are about twice as large as those of the diploid. The microspore nucleus at late prophase is also correspondingly larger in the tetraploid. The chloroplasts of the tetraploid are twice as large as those of the diploid,— a relationship also found in *Solanum* species by Winkler.

Needle-shaped spicules are found in the cells of the stem and are obtained in viscous sap exuded from a cut stem. These spicules are much longer in the tetraploid. The stomata of the tetraploid are larger than those of the diploid, and the number per square mm. of leaf surface is closely correlated with chromosome number. This relationship would be expected where cell size is related to chromosome number, because the area of the flattened epidermal cells should be closely correlated with their volume.

TABLE I

COMPARISON OF DIPLOID AND TETRAPLOID TRADESCANTIA CANALICULATA

	n	Diploid	n	Tetraploid
Volume of PMC in cu. μ — 1st Tel.	25	5,443	25	9,204
Volume of microspore in cu. μ	25	7,620	25	12,217
Volume of microspore nucleus in cu μ	25	1,150	25	2,342
Volume of chloroplasts in cu. μ	44	76	62	139
Length of spicules from stem — in μ	35	74	28	112
Stomata per sq. mm. of leaf surface (lower)	50	39	50	19
Length of stomata in μ	50	61	50	78
Number of coils in meiotic chromosome	100	5.5	100	4.5
Cytoplasmic streaming — stamen hairs — μ per sec.	25	5.4	27	4.2
Time of most frequent meiotic divisions		A.M.		P.M.
Propagation from stem cuttings		poor		good

There is little difference in the size of diploid and tetraploid plants of *T. canaliculata*, even though the cells of the tetraploid are twice as large. This means that the tetraploid has about half as many cells as the diploid. The tetraploid does not develop more rapidly than the diploid, so that rate of cell division must be much slower in the tetraploid. The differences in rate of cell division provide an opportunity for a study of certain physiological processes.

The chromosomes of somatic cells are in the form of coiled chromonemata at all stages in the cell cycle, and about 20–25 minor coils are found in metaphase chromosomes; but at meiosis a major spiral is superimposed on the minor or somatic spiral. The occurrence of major spirals at meiosis is attributed to the slower development of the meiotic cell (Sax and Sax 1935). The slower development of the tetraploid *Tradescantia* should be reflected in the degree of major coiling of the chromonemata at meiosis. A comparison of the meiotic chromosomes of the diploid and tetraploid races of *T. canaliculata* shows that the number of coils are decreased considerably in the tetraploid meiotic chromosomes (Table I).

The rate of cytoplasmic streaming in the stamen hairs was compared in the diploid and tetraploid plants. Stamen hairs were taken from freshly-opened flowers and mounted in paraffin oil. A long strand of cytoplasm was selected for observation, and the rate of streaming was determined four times for a certain length of the protoplasmic strand under observation in each cell. Two series of observations were made. In the first series, the average rate of streaming in 15 cells of the diploid was 4.2 microns per second, while in 17 cells of the tetraploid, the rate was 3.2 microns per second. A second series of observations, taken a week later, showed an average rate of 7.1 microns for 10 diploid cells and 5.9 microns for 10 tetraploid cells. Although the rate of streaming is dependent upon environmental conditions, the differences observed suggest that there is greater cytoplasmic activity in the cells of the diploid.

Cytological studies of meiotic divisions of diploid tradescantias grown in the field and in the greenhouse show that the meiotic metaphase stages are found much more frequently in the morning. Even during the winter months, few division figures are found after 10 A. M. A comparison of diploids and tetraploids grown in the field showed that in the diploids the meiotic divisions occurred most frequently in the morning, while the same stages in the tetraploid were found to be most frequent in the afternoon,— although the time of division in the tetraploid was not so limited as in the diploid.

Müntzing (1936) has shown that the polyploid forms tend to reproduce vegetatively more frequently than the diploids. This difference is found in the diploid and tetraploid forms of *T. canaliculata*. About a dozen stem cuttings were made of each type. The diploid cutting formed few roots, and only a few survived, while most of the tetraploid cuttings produced roots and survived. However, the rooting ability of the tetraploid *T. canaliculata* is not as good as that of the diploid species, *T. paludosa*.

STOMATA COUNTS

The correlation between chromosome number and size and frequency of stomata in *Tradescantia canaliculata*, suggested the possibility that the size or distribution of stomata might be used as an index of polyploidy in certain species of plants. In *Tradescantia* the relation between chromosome number and the distribution of stomata is much more marked than the relation between chromosome number and length of stomata. In Karpechenko's (1928) series of *Raphanus* × *Brassica* polyploids, the length of the stomata in mm. × 1350 was about 7 for the diploids and 9.7 for the tetraploid. Stomata counts taken from his illustrations show about 550 per square mm. for the diploid parents, 800 for the diploid F_1, and 350 for the tetraploid hybrid. There seems to be a closer correlation between chromosome number and stomata counts than between chromosome number and stomata length, and the counts are made more easily than the measurements.

Some preliminary examinations were made from species or races of known chromosome number (Table II). The stomata frequency of the diploid *Secale* was nearly twice that of the tetraploid. Among the plants in the Arboretum, *Staphylea* was chosen because it was known to have diploid, tetraploid, and hexaploid species. The stomata counts are roughly proportional to the chromosome numbers. A similar correlation was found in the diploid and tetraploid species of *Deutzia* and in the tetraploid and hexaploid species of the Caprifolium section of *Lonicera*.

These preliminary comparisons of stomata counts in diploids and polyploids indicated that stomata counts might be used to determine the presence of polyploid races in certain species, and of polyploid species in certain genera. In talking over the possibilities of this work with Dr. Edgar Anderson, we learned that he had been making stomata counts from herbarium material at the Missouri Botanical Garden, and that Dr. G. L. Stebbins had used this method at the University of California. Dr. Stebbins informs us that, in his material, the size of stomata

is a better index of chromosome number than stomata frequency. We have tried the method with a number of genera in the herbarium, and it seems to have possibilities.

TABLE II

STOMATA COUNTS FROM FRESH LEAVES

Genus	Species	Chro. No.	Stomata per sq. mm. Lower epidermis
Tradescantia canaliculata		2n	39
"	"	4n	19
Secale cereale		2n	34
"	"	4n	19
Staphylea Bumalda		2n	300
"	colchica	?	290
"	pinnata	4n	157
"	trifolia	6n	121
Deutzia gracilis		2n	382
"	reflexa	4n	168
"	scabra	5n	100
Lonicera alseuosmoides		4n	350
"	Henryi	6n	271

The number of stomata per unit of leaf surface seems to be reasonably uniform in many species, provided the counts are made from leaves of similar size and stage of maturity, and the samples taken from corresponding areas in different leaves. Long and Clements (1934) have shown that the number of stomata varies with the position on the leaf and the environmental conditions under which the leaf was developed. Accordingly, we have selected leaves only from fruiting specimens and taken the collodion peels from an area near the center of each leaf.

A modification of the collodion peel method used by Long and Clements was adopted for this work. We have used a solution of parlodion (5 per cent) in butyl acetate plus 5 per cent of butyl alcohol, as suggested by W. C. Darrah. A small drop of the solution is dropped on the lower epidermis of the leaf. If the leaf is pubescent, the pubescence may be removed by a preliminary peel, or it can often be removed with an eraser or piece of art gum. Drying of the solution is facilitated by the use of an electric hair dryer. When the edges of the parlodion begin to dry and separate from the leaf, the peel is removed

and cemented on a glass slide. The necessary data are written on the slide with a wax pencil. The stomata counts were made at a magnification of × 300 or × 450. The count for each specimen is based on an average of five records from various parts of the peel. The counts were later converted into number per square mm. of leaf surface.

The first genus selected for a study of stomata frequency in relation to polyploidy was *Malus*, a genus known to have both diploid and tetraploid species. The data obtained are shown in Table III.

TABLE III

MALUS SPECIES — STOMATA COUNTS

	Reported chr. no.	Number of specimens	Stomata per sq. mm.	
American Species				
angustifolia	4n	9	320–410 (7)	190–230 (2)
coronaria	4n	7	340–340 (2)	150–220 (5)
fusca		9	310–330 (2)	140–180 (7)
glaucescens	4n	7	310–340 (4)	180–220 (3)
ioensis	2n	6	300–390 (6)	
platycarpa		3		190–200 (3)
Asiatic Species				
baccata	2n	10	330–380 (3)	160–200 (7)
floribunda	2n	2	300–350 (2)	
Halliana		2	310 (1)	210 (1)
prunifolia	2n	3	320–430 (3)	
Sieboldii		3	320–420 (3)	
× Zumi	2n	2	330–340 (2)	

Most of the stomata counts fall into two general classes: those between 300 and 430 and those between 140 and 230. Presumably the higher counts indicate a diploid chromosome number, the lower counts a tetraploid chromosome number. Intermediate counts were found in six specimens — including *M. coronaria* and *M. Halliana* — and in the parthenogenetic triploid species *M. hupehensis*. If the stomata counts are a reliable index of polyploidy, it appears that both diploid and tetraploid races occur in *M. angustifolia*, *M. coronaria*, *M. fusca*, *M. glaucescens*, *M. baccata*, and *M. Halliana*. The tetraploid forms appear to occur more frequently in North America. There is no relation

between polyploidy and geographic distribution within a species, with the possible exception of *M. fusca*. This species extends from Alaska to California. The two apparently diploid forms were collected in Alaska and British Columbia, while only "tetraploid" forms were found in Washington, Oregon, and California.

In the closely related genus *Pyrus* only diploid species have been reported. Stomata counts from sixteen specimens, including six species, were rather variable, ranging from 120 to 320; but the average count was 190 per square millimeter.

The stomata counts of *Staphylea* species were obtained from herbarium specimens for a comparison with the counts obtained from the living plants. The average stomata frequency was 360 for the diploid, *S. bumalda;* 190 for the tetraploid, *S. pinnata;* and 220 for the hexaploid species, *S. trifolia*. While these counts are not entirely in accord with those from fresh specimens, the stomata counts in the diploid are much higher than those of the polyploids in both series of observations.

A study of stomata frequency in *Vaccinium* species indicates that both diploid and tetraploid forms occur in *V. canadense* and in *V. vacillans*. The stomata counts in the diploid and tetraploid forms are about 500 and 350, respectively. All four specimens of *V. corymbosum* examined had stomata counts ranging between 340 and 390, indicating that all were tetraploids as listed by Longley (1927). According to the stomata counts, the four specimens of *V. pennsylvanicum* examined are also tetraploids. *Vaccinium virgatum,* a hexaploid, according to Longley, had a stomata count of 290 in the one specimen examined.

The relation between polyploidy and geographic distribution suggests that many geographic races may be polyploid forms of the diploid species. We have started a series of investigations on polyploidy in relation to geographic distribution. Dr. Fernald has been very cooperative in this work, and has given us many suggestions regarding geographic races which are well represented in the Gray Herbarium.

The first species selected for study by means of stomata counts was *Gaylussacia dumosa* and its variety *Bigeloviana*. The species is found from Florida to Pennsylvania, while the variety extends from New Jersey to Nova Scotia. The stomata counts of the species ranged from 220 to 290 in seven specimens, with an average of 260; while in the variety, the stomata counts ranged from 220 to 360 in ten specimens, with an average count of 270. Apparently *G. dumosa* and its variety *Bigeloviana* have the same chromosome number; and in this case polyploidy is not involved in the taxonomic and geographic differences.

CONCLUSIONS

A comparison of diploid and tetraploid races of *Tradescantia canaliculata* shows a high degree of correlation between chromosome number and size of pollen mother cells, microspores, stomata, chloroplasts, and stomata frequency. The tetraploid has about half as many cells as the diploid forms, and a corresponding difference must exist in the rate of cell division. The number of major spirals in the meiotic chromosomes is greater in the diploid. Cytoplasmic streaming in the stamen hairs seems to be more rapid in the diploid. The tetraploid roots much better from stem cuttings.

Stomata frequency was used as an index of polyploidy in several genera. A positive correlation is found in diploid and tetraploid races of *Tradescantia* and *Secale* and in species of *Staphylea, Deutzia,* and *Lonicera.* Counts from herbarium material show some correlation between stomata frequency per square millimeter of leaf surface and known chromosome numbers of the species. If the stomata counts are a reliable index of chromosome numbers, it appears that both diploid and tetraploid races exist in certain species of *Malus* and *Vaccinium.*

DESCRIPTION OF PLATE 205

Camera lucida sketches of cells from diploid and tetraploid forms of *Tradescantia canaliculata,* and stomata size and frequency in two herbarium specimens of *Malus baccata.*

Tradescantia

Figs. 1 and 2. Anaphase of first meiotic division of diploid and tetraploid. × 800.

Figs. 3 and 4. Outlines of microspores and late prophase in diploid and tetraploid. × 800.

Figs. 5 and 6. Stomata from diploid and tetraploid races. × 250.

Figs. 7 and 8. Crystals from cells of stem in diploid and tetraploid respectively. × 400.

Malus

Stomata distribution from collodion peel.

Fig. 9. Malus baccata. Collected in Manchuria by Dorsett, no. 3609. Presumably a diploid form.

Fig. 10. Malus baccata. Collected in Siberia by Sargent in 1903. Presumably a tetraploid form.

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